Journal of Chemical Ecology, Vol. 10, No. 3, 1984

CONTACT SEX PHEROMONE IN THE TSETSE FLY *Glossina pallidipes* (Austen) Identification and Synthesis

D.A. CARLSON,[1] D.R. NELSON,[2] P.A. LANGLEY,[3] T.W. COATES,[3] T.L. DAVIS,[4] and M.E. LEEGWATER-VAN DER LINDEN[5]

[1]*USDA-ARS, Insects Affecting Man and Animals Research Laboratory Gainesville, Florida 32604*
[2]*USDA-ARS, Metabolism and Radiation Research Laboratory State University Station, Fargo, North Dakota 58105*
[3]*Tsetse Research Laboratory, University of Bristol Department of Veterinary Medicine, Langford, Bristol, England BS18 7DU*
[4]*Department of Entomology and Nematology, University of Florida Gainesville, Florida 32611*
[5]*Laboratory of Experimental Entomology, University of Amsterdam Amsterdam, The Netherlands*

(Received April 5, 1983; revised June 13, 1983)

Abstract—Adult male *G. pallidipes* attempted to copulate with decoys treated with a branched paraffin obtained from laboratory-reared female flies. The compound causing maximal response was isolated and identified as 13,23-dimethylpentatriacontane. The synthesized compound elicited increasing responses with increasing doses. This sex- and species-specific compound was always present in physiological amounts in females, as it increased from 2 μg at emergence to 10 μg per female at 14 days. It was present in wild-caught females from a wide geographical range.

Key Words—*Glossina, pallidipes*, tsetse fly, Diptera, Muscidae, pheromone, contact stimulant, branched alkane, 13,23- dimethylpentatriacontane.

INTRODUCTION

Glossina pallidipes Austen is sympatric with *G. morsitans morsitans* Westwood across large areas of eastern and central southern Africa. Both may feed on the same host animal, and both are important vectors of trypanosomiasis. Males of *G. morsitans* attempt to copulate with artificial or natural decoys treated with natural (Langley et al., 1975) or synthetic 15,19,23-trimethylheptatriacontane, while much weaker responses were seen to two

dimethyl homologs, 15,19- and 13,17-dimethylheptatriacontane (Carlson et al., 1978). The trimethylheptatriacontane released copulatory attempts from wild male *G. m. morsitans* visually attracted to decoys in the field (Langley et al., 1981). Some interspecific activity was seen, in that male *G. pallidipes* responded to decoys treated with 100 μg of the trimethyl alkane in the laboratory, although this dose is too high to be considered biologically meaningful (Huyton et al., 1980).

The presence of a sex stimulant pheromone in *G. pallidipes* was demonstrated in bioassays of males with live or dead females and female materials, including surface lipid extracts, total hydrocarbons, total paraffins, and the major 35-carbon paraffin isolated from mature females (Langley et al., 1982a; McDowell et al., 1981). In a preliminary report, we identified this material as 13,23-dimethylpentatriacontane and reported on the activity of the synthesized compound (Carlson et al., 1981). Copulatory responses were released in *G. pallidipes* males by newly emerged females, either live or killed by freezing, showing that a physiologically active quantity of sexual stimulant was present in very young females. Behavioral maturation, rather than development of cuticular stimulants, was thus considered responsible for the observation that females were most receptive at 9 days of age (Langley et al., 1982a).

The presence of female-produced sex stimulant pheromones has been demonstrated in a third species, *G. palpalis palpalis* Rob-Des. (Offor et al., 1981), and implied in a fourth, *G. austeni* Newstead (Huyton et al., 1980).

We report the analysis of cuticular paraffins from female *G. pallidipes*, and synthesis and bioassays of several potential pheromones that release sexual behavior in the male on contact.

METHODS AND MATERIALS

Wild flies for analysis were obtained as dried intact specimens shipped in capped vials (Tables 3 and 4). Larger samples were obtained as concentrates of crude ether or hexane extracts of freshly caught and chilled wild flies obtained from CO_2 plus acetone-baited traps operated in the Zambezi River Valley of Zimbabwe (Vale, 1982) (Table 2). Laboratory flies were aged for extraction in rearing cages under conditions described previously (Tables 1 and 4) (Langley et al., 1982a). Lipids from each sample were prepared for analysis by liquid chromatography on silica gel, then argentation liquid and thin-layer chromatography to obtain active paraffins (Carlson et al., 1978) for further analysis by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS).

Gas–liquid chromatography (GC) was performed on a Varian model 2100 GC using a glass column (1.8 m $\times$ 2 mm ID) packed with 3% OV-1 on 120–140 mesh Chromosorb W AWDMCS, with flame ionization detector,

and a Hewlett-Packard model 3380A recording integrator. Operating conditions were: injector, 345°; detector, 360°; helium carrier gas, 24 ml/min; and the oven was temperature programed from 150 to 325° at 6°/min. Each peak in samples of individual and pooled flies was quantitated and percentage composition determined. Each sample was reinjected on a 12 m or 25 m × 0.3 mm ID fused silica column (DB-1, J & W) together with *n*-paraffin standards of 22–40 carbons (Analabs, Inc.) and temperature programmed to 300° for determination of Kovats' indices (KI) (Kovats, 1965). Afterward, an internal standard *n*-alkane of 35 carbons was added for quantitations that were not adjusted further. Compounds were separated for bioassay by preparative GC, using a 99:1 splitter in the Varian 2100 and substituting a glass column (1.7 m × 3.2 mm ID) packed with 3% OV-1. Mass spectra were obtained with a Varian/MAT SS-200 data system and a MAT 112S electron impact mass spectrometer coupled via a jet separator to a glass column (3 m × 3.2 mm ID) packed with 3.5% OV-101 on 100–120 mesh Gas-Chrom Q. The carrier gas was helium, and the oven temperature was programed from 180 to 324° at 2°/min. The mass spectra were interpreted as previously described (Nelson, 1978; Nelson et al., 1980, 1981; Pomonis et al., 1980).

For bioassays, *G. pallidipes* or their offspring were obtained from puparia collected in the Zambezi Valley of Zimbabwe that had emerged at Bristol, England (Tables 4 and 5). Nine- to 12-day-old males from Bristol (Tables 4–6), 0- to 19-day-old males from puparia collected originally in Uganda that emerged in Amsterdam (Table 7), or flies from Amsterdam that were maintained in Bristol (Table 8) were used. Decoy objects were either *G. pallidipes* females, *G. morsitans* females, 7- to 8-day-old males freshly killed by freezing then washed with solvent, or rectangular prisms of cork (9 × 3 × 3 mm) mounted on corks. A single test male was brought into physical contact with a decoy and its response scored as 0, 1, 2, or 3 according to the vigor of its response, with 3 being the maximal copulatory response involving curving of the abdomen and engagement of the hypopygium with the decoy. Scores were added because the highest possible score ($3n = 100\%$) is the same as the score for an active female fly (Carlson et al., 1978). Decoys were baited with microliter quantitites of solutions containing extracts of females, fractions of extracts separated by liquid or preparative gas chromatography (Langley et al., 1982a), or solutions of synthetic chemicals in hexane. Bioassay conditions were as described by Huyton et al. (1980).

RESULTS

Analysis

KIs are generally used to designate natural products that are often unseparable mixtures. Names and/or Roman numerals are used for syn-

thesized materials in the text and tables. Analysis of cuticular paraffins of young females showed that the major components at KI 3563 increased by 50% from day 1 to 7.4 μg at day 8. However, the proportions of the four principal peaks at KI 3065, 3563, 3663, and 3763 did not change appreciably with age, as the mean abundances were 21, 39, 6, and 12%, respectively (Table 1). These four components comprised 78% of the paraffin complement, while the other listed minor components comprised 13% of the total, and none changed appreciably with age. Together the materials shown here comprised 91% of the total paraffins. There did not seem to be any other major materials appearing or becoming prevalent in older females, although there was more material in older females.

The average quantity of the major 3563 peak in pooled wild females from Zimbabwe was 13 μg per female, about 31% of the total paraffins (Table 2). There was variation in the quantities of paraffins found in other samples of *G. pallidipes* from various sources. However, the percentage composition of each was fairly consistent, for example, the 3563 peak ranged from 23.9 to 43% but was always the major dimethyl-branched paraffin (Table 3).

GC profiles of paraffins of wild *G. pallidipes* are compared in Figure 1. Previously, paraffins of more than 34 carbons were not found in male cuticle to any extent in virgin Bristol laboratory males (Huyton et al., 1980). In wild males of unknown history collected as adults, there were often similar small amounts of KI 3563; 0.83 μg (6% of paraffins) in Kenya males and 0.63 μg (5% of paraffins) in Zimbabwe males (Figure 1B).

Results of electron-impact mass spectral analysis of paraffins from females showed that the higher-molecular-weight compounds contained two methyl groups. Two components appeared in the KI 3563 peak. Major fragments were found at m/z 196:197 (doublet of peaks), 351, and 505 (M-15) in Kenya flies (Figure 2A). A minor isomeric component, 11,21-dimethylpentatriacontane (I) was indicated by smaller fragments at m/z 168:169, 224:225, 323, and 379. This compound contained 10- and 14-carbon alkyl chains at opposite ends of the molecule, and a 9-carbon methylene bridge between the two methyl groups. The prominent m/z 196:197 doublet, which characteristically contained more of the even-mass ion, and the prominent m/z 351 fragment were derived from the cleavage on either side of the point of methyl-branching at carbon 13 (Nelson, 1978; Nelson et al., 1980, 1981). Thus, the symmetrical structure, 13,23-dimethylpentatriacontane (II), with a 12-carbon alkyl chain at each end of the molecule and 9-carbon methylene bridge between the methyl groups, was indicated. Since some of the fragments at m/z 168:169 and 323 could be contributed by the symmetrical major isomer II, the best estimate of isomeric proportions was made by comparing the intensity of ions at m/z 351 vs. 379, present at a ratio of 13:1. Division of the 351 intensity by two because of molecular symmetry in II gave a 1:6.5 isomeric ratio of I:II in Kenya females (Table 9). Both components I and II

TABLE 1. QUANTIFICATION OF MAJOR PARAFFINS OF *G. pallidipes* FEMALES REARED IN BRISTOL

Kovats' index	Age (days)	Quantities of peaks found, μg/fly (% composition)							
		1	2	3	5	6	7	8	$\bar{X}$
3065		2.73 (22)	3.07 (18)	3.10 (20)	2.87 (25)	3.03 (20)	3.48 (19)	4.07 (22)	3.19 (21)
3165		0.19 (1)	0.22 (1)	0.28 (2)	0.13 (1)	0.17 (1)	0.22 (1)	0.20 (1)	0.20 (1)
3265		0.42 (4)	0.66 (4)	0.70 (4)	0.59 (5)	0.63 (4)	0.75 (4)	0.87 (5)	0.66 (4)
3365		0.56 (4)	0.49 (3)	0.67 (4)	0.37 (3)	0.65 (4)	0.48 (3)	0.41 (2)	0.52 (3)
3465		0.54 (4)	0.52 (3)	0.57 (4)	0.50 (5)	0.74 (5)	0.75 (4)	0.67 (4)	0.61 (5)
3563		4.51 (37)	6.22 (37)	5.99 (38)	4.23 (38)	5.92 (40)	7.45 (41)	7.43 (41)	5.96 (39)
3663		0.58 (5)	1.13 (7)	1.10 (7)	0.66 (6)	0.88 (6)	1.35 (7)	1.28 (7)	1.00 (6)
3763		1.06 (9)	2.66 (16)	2.03 (13)	1.10 (10)	1.39 (9)	2.31 (13)	2.30 (12)	1.83 (12)

TABLE 2. QUANTIFICATION OF PARAFFINS FROM POOLED *G. pallidipes* FEMALES FROM ZIMBABWE

Kovats' index	Quantities of peaks found, μg/fly (% composition)							
No. of flies:	885	715	135	349	500	367	225	$\bar{X}$
2865	0.13 (0.7)	0.25 (0.7)	0.65 (1.1)	0.26 (0.7)	0.25 (0.7)	0.48 (1.0)	0.71 (2.7)	0.39 (0.8)
2965	0.19 (1.1)	0.43 (1.2)	0.78 (1.3)	0.44 (1.2)	0.37 (1.1)	0.51 (1.1)	0.25 (1.0)	0.42 (1.1)
3065	9.67 (21.0)	11.84 (29.0)	13.34 (23.0)	10.68 (29.0)	10.16 (28.0)	11.92 (25.0)	7.33 (28.0)	10.71 (26.1)
3165	1.52 (3.2)	1.77 (3.6)	1.88 (3.2)	0.89 (2.4)	1.37 (3.8)	1.39 (2.9)	0.94 (3.6)	1.39 (3.2)
3265	2.66 (6.0)	3.16 (7.0)	3.62 (6.2)	2.30 (6.0)	2.44 (6.8)	2.82 (6.0)	1.75 (6.0)	2.67 (6.3)
3365	1.44 (3.0)	1.53 (3.0)	1.85 (3.2)	0.91 (2.5)	1.15 (3.0)	1.29 (2.7)	0.67 (2.6)	1.26 (2.9)
3465	1.48 (3.0)	1.38 (2.0)	1.91 (3.3)	0.94 (2.5)	0.89 (2.5)	1.16 (2.4)	0.60 (2.3)	1.19 (2.6)
3563	11.92 (26.0)	13.73 (29.0)	17.08 (30.0)	12.24 (34.0)	12.20 (34.0)	15.03 (32.0)	8.75 (34.0)	12.99 (31.3)
3663	2.60 (6.0)	2.60 (4.0)	3.63 (6.0)	1.59 (5.0)	1.75 (5.0)	2.18 (5.0)	1.29 (5.0)	2.23 (5.1)
3763	5.6 (12.0)	6.11 (11.0)	6.83 (12.0)	4.15 (10.0)	4.40 (12.0)	5.59 (12.0)	3.27 (13.0)	5.13 (11.7)

TABLE 3. QUANTIFICATION OF MAJOR PARAFFINS OF LABORATORY-REARED OR WILD *G. pallidipes* FEMALES FROM DIFFERENT COUNTRIES

	Quantities of peaks found, μg/fly (% composition)									
	Age (days):	1[a]	60[a]	110[a]	1–5[b]	70[b]	77[b]	Wild[c]	28–35[b]	Wild[d]	Wild[e]
Kovats' index	No. of flies:	1	1	1	10	10	10	20	10	10	2
2865		0.55 (2.4)	0.70 (1.1)	-	0.48 (2.8)	0.27 (0.8)	0.17 (0.7)	0.47 (1.9)	0.21 (0.6)	0.64 (1.9)	0.70 (3.3)
2965		0.28 (1.2)	0.48 (0.8)	0.67 (0.8)	0.27 (1.6)	0.29 (0.9)	0.19 (0.8)	0.36 (1.5)	0.42 (1.3)	0.46 (1.4)	0.89 (4.2)
3065		4.03 (18.0)	10.10 (17.0)	14.46 (26.0)	3.29 (19.4)	6.41 (19.4)	4.58 (18.8)	4.43 (18.0)	5.34 (16.5)	10.62 (31.9)	4.08 (19.2)
3165		0.41 (1.8)	1.04 (1.7)	0.75 (1.3)	0.36 (2.1)	0.69 (2.1)	0.55 (2.2)	1.51 (6.1)	0.79 (2.4)	0.53 (1.6)	1.12 (5.3)
3265		0.95 (4.0)	3.44 (5.6)	4.15 (7.5)	0.79 (4.6)	1.99 (6.0)	2.05 (8.4)	1.48 (6.0)	2.65 (8.2)	2.34 (7.0)	1.38 (6.4)
3365		0.89 (3.9)	1.69 (2.8)	1.97 (3.6)	0.79 (4.7)	0.92 (2.8)	0.85 (3.5)	1.13 (4.5)	0.93 (2.9)	0.41 (1.2)	1.02 (4.8)
3465		0.78 (3.4)	2.10 (3.5)	1.80 (3.2)	0.50 (3.0)	1.22 (3.7)	1.24 (5.1)	0.70 (2.9)	1.33 (4.1)	0.57 (1.7)	0.79 (3.7)
3563		9.26 (40.0)	26.17 (43.0)	19.57 (35.3)	5.84 (34.5)	12.90 (39.0)	9.05 (37.1)	7.83 (31.8)	12.12 (37.4)	7.97 (23.9)	5.33 (24.5)
3663		0.90 (4.0)	3.45 (6.0)	1.87 (3.3)	0.72 (4.2)	2.03 (6.1)	1.65 (6.7)	1.10 (4.5)	2.48 (7.6)	1.28 (3.8)	1.29 (6.0)
3763		1.51 (7.0)	7.38 (12.0)	3.47 (6.2)	1.52 (9.0)	3.70 (11.2)	2.86 (11.7)	2.38 (9.7)	4.89 (15.1)	4.51 (13.5)	2.40 (11.3)

[a]Kenya/Austria.
[b]Uganda/Amsterdam.
[c]Kenya (Kiboko).
[d]Zambia (Kakumbi).
[e]Mozambique (Muabsa).

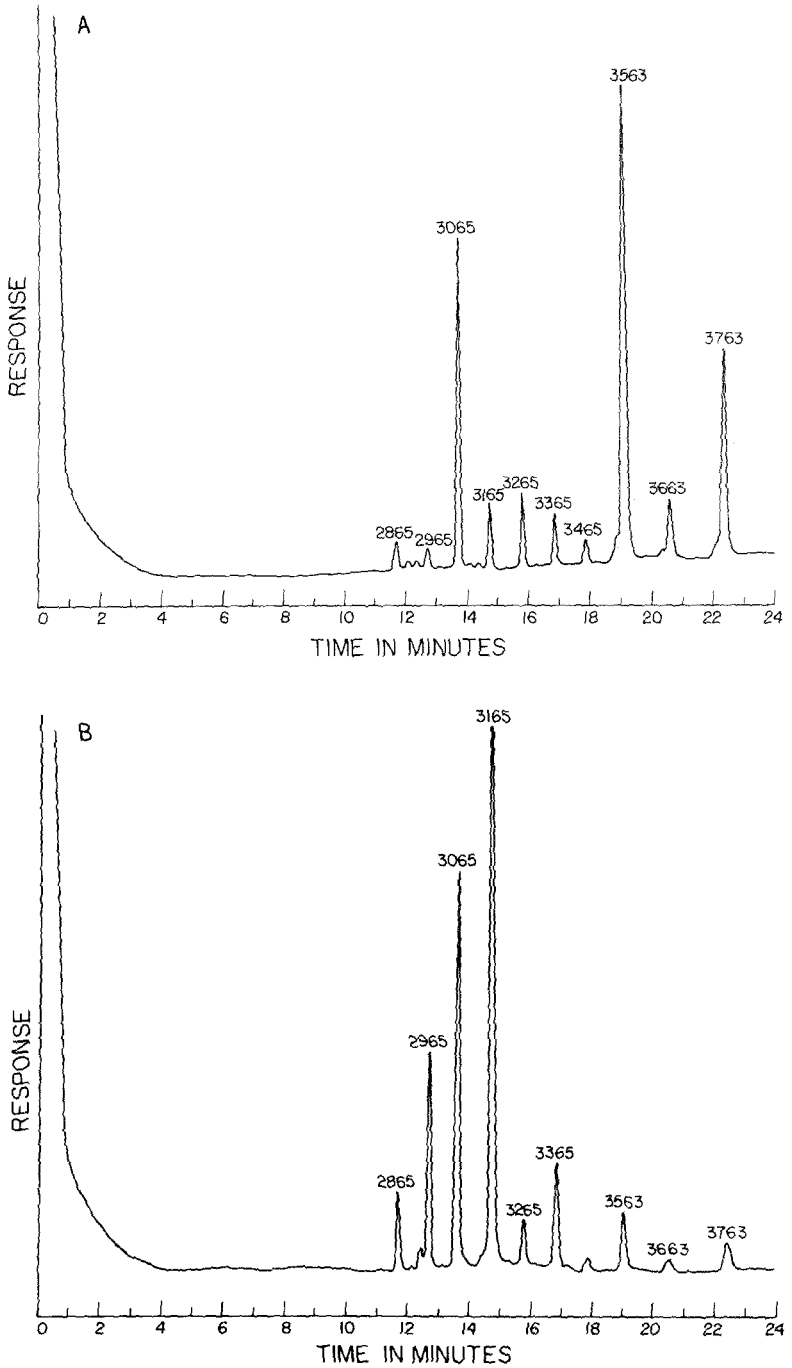

FIG. 1. GC of cuticular paraffins from *G. pallidipes:* (A) Kenya wild females (B) Zimbabwe wild males.

were found in the 3563 peak of all flies analyzed by GC-MS and were present in the following proportions: 1:5 in Bristol flies, trace:1 in Mozambique and Zambia flies, 1:8 in Uganda/Amsterdam flies, and 1:6 and trace:1 in two samples of Zimbabwe flies.

The quantity of II present in the cuticle of females was estimated by combining GC data with isomeric proportions obtained by GC-MS (Table 9). Thus laboratory 8-day-old Bristol females had 5.9 μg (80% of KI 3563 peak of 7.4 μg, Table 1), and Zimbabwe females had 10.8 μg (83% of $\bar{x}$ 13 μg) to 12.3 μg (95% of 13 μg, Table 2). Similarly, levels of II found in wild females from four other countries (Table 3) lay between these values, except for 60- or 110-day-old laboratory Kenya/Austria flies that had 26 μg and 19 μg. For purposes of comparison, an intermediate quantity of 10 μg per female of natural II was assigned. Material that eluted as a shoulder at KI 3579 on the trailing edge of the KI 3563 peak appeared to contain 11,15,21- and 13,17,23-trimethylpentatriacontane and constituted about 4% of the total complement of paraffins from several samples, including Zimbabwe females (Table 9, Figure 1).

Two homologous isomers were present in the KI 3663 peak, 12,22- (III) and 13,23-dimethylhexatriacontane (IV), present in the various ratios (Table 9). The structures were consistent with prominent fragments found at m/z 182:183, 224:225, 337, 379, and 519 (M-15) for III, and 196:197, 210:211, 351, and 365 for IV (Figure 3).

The major isomer present in the 3763 peak of all flies, consistent with the prominent fragments found at m/z 196:197, 224:225, 351, 379, and 533 (M-15) was structure V, 13,23-dimethylheptatriacontane (Figure 4). It was a homolog of the 3563 compounds, having two more methylene units added to the terminal chain.

Other dimethyl compounds with nine methylene units between the branch points identified by GC-MS include 12,22-dimethyltetratriacontane, three compounds present with 38-C backbones, (12,22-, 13,23-, and 14,24-dimethyloctatriacontane), and two compounds present with 39-C backbones (13,23- and 15,25-dimethylnonatriacontane).

Retention indices for synthetic and natural dimethylpentatriacontanes on a nonpolar fused silica column were: natural and synthetic I and II, KI 3563; 13,17-dimethylpentatriacontane, KI 3554; 15,19-dimethylpentatriacontane, KI 3555.

Synthesis of paraffins

1-Bromo-9-[triphenylphosphonio]nonane Bromide (VI). Triphenylphosphine (1.1 equiv, 2.01 g) and 1,9-dibromononane (2.0 g, 7.0 mmol) were brought to reflux in 20 ml of xylene. The mono-Wittig salt began to appear as an oil in 1 hr. GC analysis (3% OV-1, TP 150–325° at 12°/min, T_r dibromononane = 3.2 min) indicated greater than 90% completion in 4.5

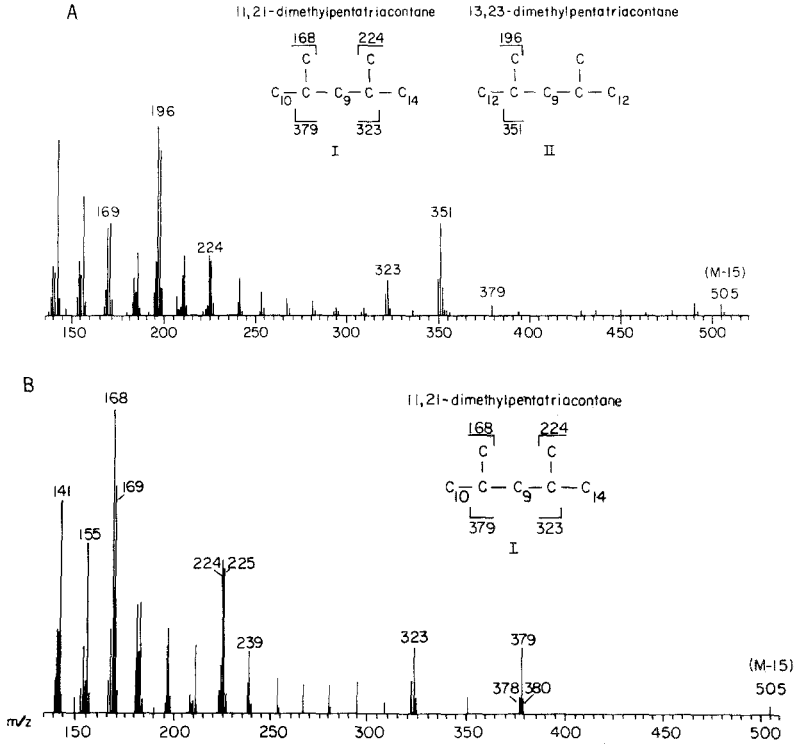

FIG. 2. Mass Spectrum of: (A) KI 3563 peak from *G. pallidipes* females from Kenya, (B) synthetic 11,21-dimethylpentatriacontane, (C) synthetic 13,23-dimethylpentatriacontane.

days. Since the first step was pseudo-second order in either reagent, it was followed by plotting the reciprocal of the concentration of either reagent, as shown by GC. The solvent was removed in vacuo on a rotary evaporator, the product taken up in $CHCl_3$, then precipitated as a viscous oil by addition of diethyl ether. Repeated trituration with ether afforded a colorless, hygroscopic powder which was dried overnight in vacuo to give 3.2 g (83% from the dibromide). The product was further purified by crystallization from methylene chloride/THF, melting with loss of solvent at 129–130°.

The identity of VI was confirmed by the proton NMR spectrum ($CDCl_3$): 7.8 ppm (multiplet = m) 15H, aromatic; 3.7 ppm (m) 4H, $Ph_3P^+CH_2$ and CH_2Br; 2.4 ppm (m) 2H, $Ph_3P^+CH_2CH_2$; 1.2–1.9 ppm (m) 14H, methylene envelope.

1,9-Bis(triphenylphosphonio)nonane Dibromide (VII). Triphenylphosphine (2.4 g, 1.1 equiv) and 4.6 g of VI (8.4 mmol) were combined in a dry 100-ml flask. The flask was purged with argon, equipped with a glass Tru-

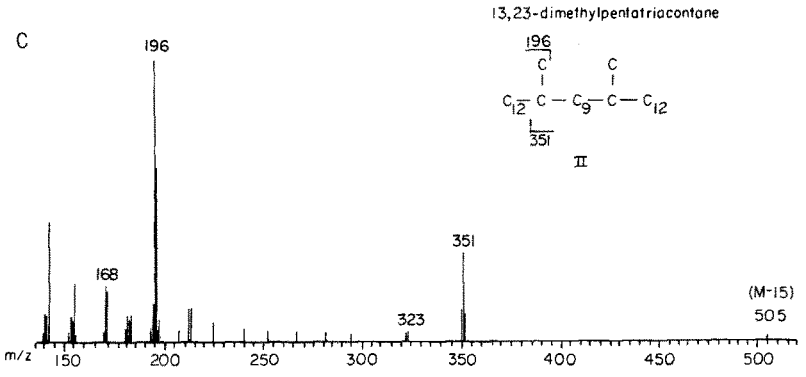

FIG. 2. Continued.

bore stirrer, and heated for 2 hr in a 230–260° sand bath (Mondon, 1957). The melt became viscous, but did not solidify. Upon cooling, the resultant hard, glassy product was dissolved in $CHCl_3$, and the bis-Wittig salt precipitated with ether. Repeated trituration with ether afforded 6.6 g of an off-white powder. NMR analysis indicated the product contained about 20% of the mono salt. Crystallization from methylene chloride–THF gave 6.0 g (74% yield of the pure VII, based upon VI) of the pure bis-Wittig Salt, mp 290–295° (loss of solvent 140–145°).

11,21-Dimethylpentatriaconta-11,20-diene. A 0.1 g (1.2 mmol) portion of bis-Wittig VII in 30 ml of freshly distilled THF was purged with nitrogen and chilled to 5° in an ice bath. Butyllithium was added from a syringe until a faint, persistent yellow color was obtained, then 2 equiv (3.5 ml, Aldrich, 1.6M in hexane) of *n*-BuLi were added dropwise over 10 min below 10°. Immediately after addition, an equal volume of freshly distilled hexamethylphosphoramide (HMPA, bp 90°/2.5 mm, from CaH_2, Aldrich) was added. No change was noted in the brown-orange color of the reaction mixture. To the ylid solution was added 0.868 equiv each of 2-hexadecanone and 2-dodecanone (Chemical Samples, 250 and 192 mg, respectively). The reaction mixture was maintained 1 hr below 10°, 1 hr at 25°, and 2 hr at 65° before being cooled, diluted with water, and the products extracted three times with hexane. The combined hexane phases were washed with brine, dried over sodium sulfate, and concentrated to give 0.45 g of oil. Chromatography on activated silica gel (20 g, V_0 = 55 ml) gave 50 mg of nonpolar material that eluted at 1–1.2 V_0 with hexane. To recover the olefins, chromatography on 20% $AgNO_3$-impregnated silica gel (10 g, Applied Science, Hi-Flosil /Ag, 60–200 mesh, V_0 = 23 ml), eluting with $4V_0$/hexane, then $4V_0$/hexane: 2% ether, gave 2.4 mg (0.4% yield) of a diolefinic fraction at 2–2.7 V_0 of the latter [TLC: R_f = 0.45; 3% ether–hexane, 15% $AgNO_3$-impregnated silica gel (Analabs, 250 μm, 20 cm), where (*E*)-9-tricosene R_f = 0.72 and (*Z*)-9-

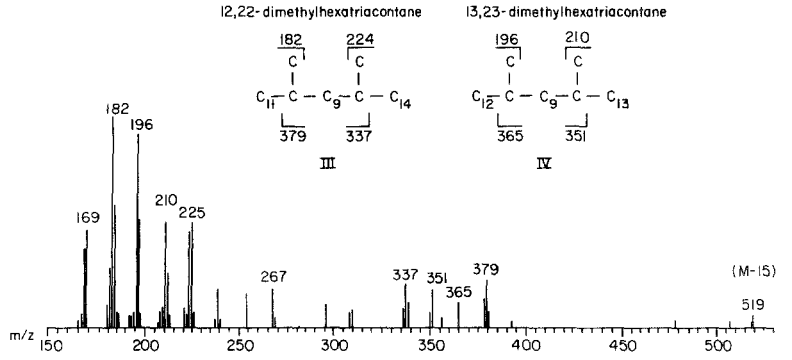

FIG. 3. Mass Spectrum of KI 3663 peak from *G. pallidipes* females from Kenya.

tricosene $R_f = 0.64$.]. The expected mixture of diolefins was produced: 11,21-dimethylhentriaconta-11,20-diene (0.64 mg, three GC peaks at KI 3100, 3148, and 3200), 11,21-dimethylpentatriaconta-11,20-diene (1.10 mg, three GC peaks at KI 3500, 3547, and 3600), and 15,25-dimethylnonatriaconta-15,24-diene (0.64 mg, three GC peaks at 3690, 3737, and 3800).

11,21-Dimethylpentatriacontane (*I*). The diolefin mixture was hydrogenated over 200 mg of prereduced catalyst (Alfa, 5% Pt on carbon) at 40 psi for 48 hr with magnetic stirring in 2 ml hexane, to give ca. 80% reduction. The sample was charged with 100 mg of fresh catalyst and hydrogenation continued overnight. The catalyst was filtered and the concentrated mixture chromatographed on 5 g $AgNO_3$-impregnated silica gel (V_o = 25 ml), eluting with hexane (1–1.75 V_o). The expected mixture of three paraffins was produced: 11,21-dimethylhentriacontane (44%, KI 3267), 11,21-dimethylpentatriacontane (I, 46%, KI 3563), and 15,25-dimethylnonatriacontane (10%, KI 3965).

The desired paraffin (I) (ca. 0.4 mg) was isolated by preparative GC. The

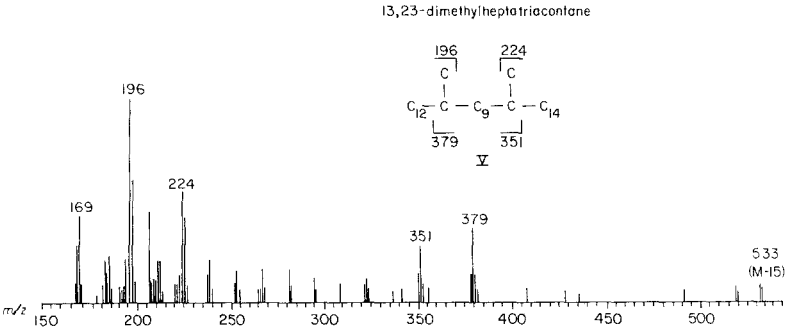

FIG. 4. Mass Spectrum of KI 3763 peak from *G. pallidipes* females from Kenya.

spectrum of synthetic I showed the expected major fragments at m/z 168:169 (100:76%), 224:225 (50:47%), 323 (21%), 379 (21%), and 505 (3%, M-15). Small excesses of ions were seen at m/z 140 and 169 but were not indicative of major homologs (Figure 2B).

13,23-Dimethylpentatriaconta-13,22-diene. Two individual preparations of VII were made using acetonitrile and benzene and combined as a viscous glass that contained some VI. The mixture (31.6 g, 38.9 mmol if all VII) was combined with 175 ml of HMPA in a dry 500-ml 3-neck flask in the dry box. The flask was purged with nitrogen, stirred magnetically, and cooled to 7° during dropwise addition from a syringe of 3 equiv (1.5-fold excess) of 2.4 M *n*-butyllithium (48 ml, 115 mmol) in hexane to give a clear, dark red solution. The ylid solution was held below 10° during addition of 2-tetradecanone (24.5 g, 115 mmol) (Chemical Samples) in 30 ml HMPA. After 3 hr, the flask was allowed to warm to room temperature. The next day the reaction mixture was diluted with 200 ml of water, the organic layer separated with the aid of more hexane, washed with water and brine, dried, and concentrated to give 57 g dark oil. Volatiles were removed from the crude product by vacuum distillation at 186° at 0.5 mm, leaving 18.3 g of brown oil. Chromatography on silica gel of a 1.9-g portion of the crude product gave 0.08 g of nonpolar material eluted at 1.0–1.2 V_o with hexane. It consisted of a mixture containing 13,23-dimethylpentatriaconta-13,22-dienes, which showed three GC peaks at 3500, 3548, and 3600.

13,23-Dimethylpentatriacontane (II). Two crude diolefin preparations (55 g) were added to 50 ml propionic acid in a 100-ml stainless steel bomb together with 1 g of prereduced 5% platinum on charcoal catalyst. The bomb was pressurized to 120 psi and heated for 24 hr at 150–230° with shaking for 96 hr. GC analysis of a cleaned-up hexane-soluble aliquot indicated that the three diolefin peaks had coalesced into one, at KI 3563. The cooled contents of the bomb were then poured into water in a separatory funnel together with bomb washings and partitioned against hexane. The organic layer was washed with water and brine, filtered, dried, and the solvent removed to leave 14 g of viscous brown oil.

To recover II, the crude material was divided into four parts, and each was filtered through a 2 × 45-cm column of silica gel with hexane. The first paraffin-containing fractions (1–2 V_o) were combined and the solvent removed to yield 1.2 g (4.0%) of white solid paraffin, 13,23-dimethylpentatriacontane (II). Argentation TLC using hexane showed that no unsaturated materials were present. GC analysis showed the major peak (KI 3563) comprised 96% of the material present, and several paraffins with lower molecular weight comprised the impurities. The GC retention was the same as that for the natural product. The mass spectrum of synthetic II showed the expected major fragments as m/z 196:197 (100:61%), 351 (31%), and 505 (2%, M-15). Another even–odd doublet was visible at m/z 168:169 (19:17%) from cleav-

age of the terminal alkyl chain of 12 carbons. Fragment ions were also present at m/z 210:211 (9:8%) and 323 (3%) that are real but not explained (Figure 2C).

Bioassay

Wingless, immature (0–1 days old) *G. pallidipes* females killed by freezing were nearly as stimulatory as older (9-day-old) females which released 80–100% copulatory responses in test males (Table 6) Langley et al., 1982a). Live or dead *G. pallidipes* males or *G. morsitans* male decoys never released sexual behavior in test males, allowing use of dead males as convenient decoys for bioassay of test materials for release of sexual behavior.

Using flies which emerged from field-collected puparia in Zimbabwe, a crude extract of females elicited from 37 to 70% responses from test males, and there was little indication of a dose-response relationship at doses of 0.1–10 female equivalents (FE) (Table 4). Similar responses were obtained

TABLE 4. SEXUAL RESPONSES OF TEST MALE *G. pallidipes* TO EXTRACTS OR FRACTIONS OF EXTRACTS OF CUTICLES OF FEMALES

Extract or fraction	Dose (FE)	N	Test male responses[a]				Response (%)
			0	1	2	3	
1. Virgin lab, extract (7 day)[b]	0.1	10	2	2	5	1	50
	0.2	10	3	2	4	1	43
	0.5	10	2	2	5	1	50
	1	10	2	1	3	4	63
	2	10	2	0	3	5	70
	5	10	2	1	6	1	53
2. Virgin lab, extract (7–9 days)[b]	10	5	0	3	2	0	47
	1	10	1	7	2	0	37
3. Hydrocarbons[c]	1	10	2	4	2	2	47
Cholesterol esters	1	10	10	0	0	0	0
Methyl esters	1	10	10	0	0	0	0
Triglycerides, free fatty acids	1	10	10	0	0	0	0
4. Wild caught, extract	5	20	3	4	10	3	55
5. Hydrocarbons[d]	4	6	0	1	3	2	72
Cholesterol esters	4	6	6	0	0	0	0
Triglycerides	4	6	6	0	0	0	0
Free fatty acids	4	6	6	0	0	0	0

[a]0 = no response; 1 = mounting, gripping, and arresting of male on decoy; 2 = characteristic copulatory movement with orientation to the copulatory position, abdomen curving; 3 = flexing of genetalia.
[b]Reared from wild-collected pupae of Zimbabwe origin.
[c]Fractions from virgin lab, extract.
[d]Fractions from wild-caught extract.

with 5 FE of an extract of wild-caught females (55%) and 5 FE of an extract of females emerged in the laboratory (53%). Of fractions obtained by liquid absorption chromatography on silica gel, only the hydrocarbons elicited responses from test males, and these were similar in magnitude to the responses elicited by crude extracts. Further testing showed that only the cuticular paraffins, separated by argentation chromatography from the hydrocarbon fraction, released male responses (Table 4).

Preparative gas chromatography was then employed to separate the cuticular paraffins. Bioassay of these materials showed that optimal activity (41% response) was associated with the KI 3563 peak, while some activity was associated with material collected as KI 3465, 3663, and 3763, perhaps because they are structural homologs of the KI 3563 materials (Table 5). A dose–response relationship could not be established and responses to a range of doses from 1.1 to 11.3 FE were therefore pooled (Table 5).

The two compounds present in the KI 3563 peak were synthesized and, using dead male *G. morsitans* as decoys, their ability to elicit sexual responses from test males of Zimbabwe origin was compared with that of crude cuticular extract, freshly killed *G. pallidipes* females, solvent-washed females, or male *G. morsitans* alone. Only 13,23-dimethylpentatriacontane (II) elicited test male responses including copulatory attempts (level 3). Furthermore, there was a strong indication of a dose–response relationship, although the maximum percentage response at 8 FE was no better than that obtained from 2 FE of crude extract and was lower than that obtained when dead females were used as decoys (Table 6).

TABLE 5. SEXUAL RESPONSES OF 9-DAY-OLD TEST MALE *G. pallidipes* TO ISOLATED PARAFFINS FROM FEMALES DOSED ONTO DEAD MALE DECOYS[a]

Test sample (Kovats' index)	N	Test Male Responses				Total	Response (%)
		0	1	2	3		
2865	5	5	0	0	0	0	0
2965	5	5	0	0	0	0	0
3065	25	25	0	0	0	0	0
3165	16	16	0	0	0	0	0
3265	16	16	0	0	0	0	0
3365	16	16	0	0	0	0	0
3465	16	15	0	1	0	2	4
3563	48	15	9	19	4	59	41
3663	43	37	0	3	0	6	5
3763	42	35	4	3	0	10	8

[a]Responses scored as 0, 1, 2, 3, and summed. Samples collected by preparative GC from female *G. pallidipes* surface hydrocarbons. Dose ranges from 1.1 to 11.3 FE/decoy. R(%) = total response score/3N.

TABLE 6. SEXUAL RESPONSE OF *G. pallidipes* MALES OF ZIMBABWE ORIGIN TO DEAD FEMALES OR DECOYS DOSED WITH SYNTHETIC 13,23-DIMEYHYLPENTATRIACONTANE (II)[a] OR CRUDE EXTRACT OF *G. pallidipes* FEMALES

Test sample[b]	N^c	Test male responses 0	1	2	3	Total	Response (%)	FE[d]	Amount (μg)
G. pallidipes (9 day old)	20	0	2	6	12	50	83.3		
G. pallidipes (washed)	20	16	4	0	0	4	6.7		
G. morsitans (washed)	20	20	0	0	0	0	0	2	
Crude extract	20	3	3	9	5	36	60.0	2	
II	20	5	3	9	3	30	50.0	2	20
II	10	10	0	0	0	0	0	0.1	1
	10	8	2	0	0	2	6.7	0.2	2
	10	8	0	2	0	4	13.3	0.5	5
	10	5	2	3	0	8	26.6	1	10
	10	4	2	2	2	12	40.0	2	20
	10	2	2	3	3	17	56.6	5	50

[a]I showed no responses in any tests tested alone.
[b]All samples treated onto dead 19-day virgin *G. morsitans* males except as noted.
[c]Five male decoys used at each dose.
[d]Based on estimated 10 μg II per female (see text).

TABLE 7. SEXUAL RESPONSE OF *G. pallidipes* MALES OF VARIOUS AGES OF UGANDA ORIGIN TO CORK DECOYS DOSED WITH 13,23-DIMETHYLPENTATRIACONTANE (II) AND DEAD *G. pallidipes* FEMALES (8–10 DAYS OLD), TEST CONDUCTED IN AMSTERDAM

Age of males (days)	Dead females	n	Response of test male (%) Compound II (μg) 25	50	75	100	150	N
0	2	14	0	0	0	0	0	10
1–3	26	14	0	0	0	0	0	6
4–5	60	14	8	11	39	39	33	24
6–7	58	12	13	42	78	69	58	15
8–10	89	12	72	58	84	87	77	23
11–12	83	8	86	83	98	100	100	14
13–14	97	13	97	97	100	100	100	12
15–17	92	8	94	100	100	100	100	6
18–19	79	8	100	88	100	100	100	8

TABLE 8. SEXUAL RESPONSE OF 12–14-DAY-OLD *G. pallidipes* MALES OF UGANDA ORIGIN TO CORK DECOYS DOSED WITH 13,23-DIMETHYLPENTATRIACONTANE (II) AND CRUDE EXTRACT OF *G. pallidipes* FEMALES

		Test male responses					Response		Amount
	N^a	0	1	2	3	Total	(%)	FE	(μg)
Crude extract	6	6	0	0	0	0	0	0.2	3[b]
	6	4	1	0	1	4	22.2	0.6	7
	6	3	0	0	3	9	50.0	1.3	13
	6	2	1	0	3	10	55.5	2.6	27
	6	2	0	1	3	11	61.1	6.7	67
II	6	3	1	0	2	7	38.8	0.2[b]	2
	6	2	1	0	3	10	55.5	0.5	5
	6	1	0	0	5	15	83.3	1.0	10
	6	0	0	0	6	18	100.0	2.0	20
	6	0	0	0	6	18	100.0	5.0	50

[a]Two decoys per dose and three test males per decoy (Amsterdam–Bristol colony).
[b]Based on 10 μg II per fly (see text).

Male *G. pallidipes* of Uganda origin, which have been in laboratory culture in Amsterdam for several years, were used to bioassay synthesized II using cork decoys (see Methods and Materials). Results (Table 7) show clearly that the responsiveness of males increased with age and that during the first week of adult life males of this species do not possess the ability to respond predictably to increasing doses of II. Older males responded maximally to the higher doses, but even the lowest dose tested was in excess of the amount of material which occurs naturally on or in the cuticle of a female fly. These experiments were therefore repeated using 12- to 14-day old Uganda–Amsterdam–Bristol males, and a comparison was made between crude extract of cuticle and compound II at doses of 2–50 μg. The results (Table 8) showed that 83.3% copulatory responses were obtained to 10 μg of II and obligatory (100%) copulatory responses were obtained to 20 and 50 μg, demonstrating its superiority over crude extract.

DISCUSSION

Sexual responses of adult males of *G. pallidipes* were elicited only by natural 35-carbon backbone dimethyl paraffins in the surface cuticular waxes of females. Since formal evidence was obtained for the existence of two major compounds in the KI 3563 fraction, both compounds were synthesized, and in

bioassays only the latter compound elicited sexual responses from males; no synergism was apparent.

Although the amount of 3563 paraffin increases in females as they age, there is sufficient material on or in the cuticle at emergence to elicit almost obligatory copulatory responses from test males towards live or fresh-killed females used as decoys (Langley et al., 1982a). Hence, we conclude that maturation of sexual behavior in females and perhaps also in males of *G. pallidipes* is more important than changing levels of a stimulatory pheromone in regulating the sexual behavior of this species.

Since quantities of natural II ranged from ca. 6 to 12 μg per female in most flies, a level of 10 μg of II was used to compare its activity with that of crude extract (Tables 4, 6, and 8). The inconsistencies observed and the lack of a clear dose–response relationship, except in the case of II in Table 8 where 100% responses of test males were achieved, can probably be explained as follows.

Tsetse emerging from field-collected puparia often behave differently from those which have been in laboratory culture for some time. Differences in reproductive behaviour also are known to exist among separate populations of *G. pallidipes* in the wild (Van Etten 1981). Using *G. pallidipes* emerging from field-collected puparia of Zimbabwe origin, test males never produced obligatory (100%) copulatory responses to extracts of synthetics in bioassay (Tables 4 and 6) and only produced 83.3% response to killed conspecific females (Table 6). Table 7 shows clearly that 100% responses to II were elicited in test males of Uganda origin that had been in laboratory culture for some time, but that the responsiveness to a given dose increased with age, and the doses required to elicit 100% response were in excess of the amounts known to exist on the cuticle of a female fly. Nevertheless, from Table 8, it is clear that synthetic II is superior to crude extract, and using test males of Ugandan origin (12–14 days old) an ED_{50} of around 5μg can be determined.

Ideally an ED_{95} of around 5 μg would put the assignment of II as the sex pheromone of *G. pallidipes* beyond doubt. However, the bioassay system was developed for use initially with *G. morsitans* (Huyton et al., 1980), and stimuli other than chemical may be of relatively greater importance in the initiation of mating behavior in *G. pallidipes* than in *G. morsitans*. The superiority of II over crude extracts may be due to the masking effect of other paraffins present in the extract (Coates and Langley, 1982).

Alternatively, some component synergistic with II may be missing. Synthetic compound I does not appear to be a contributor to activity, but the KI 3579 material, difficult to separate by preparative GC, contains 13,17,23-trimethylpentatriacontane. This candidate structure combines the features of II and of 13,17-dimethylpentatriacontane which is equally active in bioassays (Langley, unpublished data), but which was erroneously identified as the sex pheromone of *G. pallidipes* females (McDowell et al., 1981).

The quantities of II present in females from Bristol (ca. 6 μg), wild females from Zimbabwe (10.8–12.3 μg), and other African countries (5.3–7.9 μg) were in marked contrast to the 80 μg of pheromone per fly quoted by McDowell et al. (1981). The latter observation was probably due to the inclusion of internal lipids in the weighed extract. Inaccurate quantitation of candidate pheromone compounds can lead to spurious conclusions from bioassay data concerning their biological significance.

The element of symmetry in the structure of biologically active sexual stimulants for *Glossina* is therefore again proposed. It is interesting to note that the most active synthetic compounds found for *G. morsitans*, 15,19,23-trimethylheptatriacontane (Carlson et al., 1978) and 15,19-dimethyltritriacontane in *G. austeni* (Huyton et al., 1980), are symmetrical structures, as is II. The nonsymmetrical structures proposed by other workers (McDowell et al., 1981), 13,17-dimethylpentatriacontane (major) and 15,19-dimethylpentatriacontane (minor), are not supported by the mass spectra published in that paper. Characteristic fragment ions that should be present for McDowell's proposed major compound do not appear at m/z 280:281 and 267, and fragment ions that should be present for the minor compound at m/z 252:253 and 295 are again missing from the published spectrum. These compounds with 3-methylenes between the branch points are present in the KI 3550–3555 peak in *G. morsitans* females, but not in any of the females of *G. pallidipes* we have investigated to date. Our magnetic sector mass spectra of paraffins from females from laboratory colonies (Zimbabwe–Bristol and Uganda–Amsterdam) or wild females captured in Kenya, Mozambique, Zambia, and Zimbabwe showed remarkable consistency (Table 9) in that 11,21- and 13,21-dimethyl structures having 35-, 36-, and 37-carbon backbones were the only ones found.

The stereochemical character of structures discussed here may play a role in their function, since a symmetrical molecule with two asymmetric centers such as II may have a *meso* form and a racemic pair of enantiomers: *R,S*-, *R,R*-, and *S,S*-. Because natural II, if optically active, may contain only one of the enantiomers, the unnatural enantiomer or the *meso* form may either be inhibitors of response or passive diluents. Synthesized stereoisomers of *G. morsitans* 17,21-dimethyl pheromone components showed increased biological response in only the *meso* C_{37} dimethyl stereoisomer (Ade et al., 1980), while all the stereoisomers of 15,19,23-trimethylheptatriacontane were found to be equally active (Helmchen and Langley, 1981). Although no stereochemical preference was shown by *G. morsitans*, there is no reason to suppose that this situation obtains for other species. Molecular models indicate that there is sufficient conformational and rotational freedom that in a dimethyl compound having three or nine methylene-bridge carbons, the two methyl groups may be oriented to nearly coplanar positions regardless of whether an *R,R*-, *S,S*-, or *meso* compound has been constructed. It is thus impossible to predict which optical isomer would be most active, or

TABLE 9. POSITION OF METHYL BRANCHING AND PROPORTIONS OF ISOMERS IN PARAFFINS OF FEMALE *G. pallidipes* FROM DIFFERENT COUNTRIES

KI	Position of methyl branching[a]	Proportions of isomers seen[b]						
		Bristol	Kenya	Mozambique	Uganda	Zambia	Zimbabwe	Zimbabwe
3363	11,15-; 13,21-	1.7:1	2.7:1	1.6:1	1:1.6[c]	2.2:1	1.6:1	1:2
3463	11,21-; 12,22-	T:1	1:3	T:1	T:1	T:1[d]	T:1	1:5
3563	11,21-; 13,23-	1:5	1:6.5	1:11	1:8	T:1	1:6	T:1
3579	11,15,21-; 13,17,23-	U[f]	U	U	2.6:1	1:1	2:1	2:1
3663	12,22-; 13,23-	2.1:1	1.4:1	1:1.6	1.7:1	1.4:1	1:1	1.6:1
3763	11,21-; 13,23-[e]	T:1	T:1	T:1	T:1	T:1	T:1	T:1

[a]KI 2865, 2965, and 3065 peaks were universally 2-methyl, as each showed a small M, large M-15, and base peak M-43.
[b]Ratio of fragment intensities used to establish proportions of isomers: (Pk Ht/2) m/z 351 ÷ Pk Ht m/z 379.
[c]Trace of 9,X- in Uganda/Amsterdam flies.
[d]Trace of 2-Me.
[e]T = trace, 5%.
[f]U = trace of trimethyl-branched paraffin present, but branching positions could not be determined.

what advantage one would have over the other, especially since the conformation of receptors is unknown.

Identification and synthesis of the sex pheromone of *G. m. morsitans* led to attempts to exploit its properties for tsetse control in Tanzania (Langley et al., 1981) and Zimbabwe (Langley et al., 1982b). In the latter effort, observations led to the conclusion that the sexual behavior of *G. pallidipes* is different from that of *G. m. morsitans* in that males of the former are not visually stimulated to make contact with decoys on host models as are males of the latter. Also, *G. pallidipes* have not been observed mating in the field in Zimbabwe, in contrast to *G. m. morsitans*, which is frequently observed mating on or around host animals (G. Vale, personal communication). Although *G. pallidipes* has been observed mating in the field in eastern Africa (D. Turner, personal communication), laboratory observation has shown marked differences in behavior between flies from two different regions of Kenya. The two populations differed from each other in copulation time, pupal weight, and age at which the first larva is produced. Both exhibited rearing difficulties. These behaviors are thought to have a genetic basis and to illustrate population diversity in this species (Van Etten, 1981). In spite of such differences in the sexual behavior of *G. pallidipes* from different regions, it appears from the present results that the species uses a single compound as its sex pheromone. Development of control strategies for *G. pallidipes* involving the use of its sex pheromone will undoubtedly require a greater understanding of its reproductive behavior in the field.

Acknowledgments—This paper reports the results of research only. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA. We thank S. King for excellent technical assistance, C. Fatland and A.T. Proveaux for mass spectra, G. Vale and the government of Zimbabwe for assistance and the use of their facilities. We thank P.E. Sonnet for samples of synthetic 13,17- and 15,19-dimethylpentatriacontane. We thank the following for samples: G. LaBrecque, International Atomic Energy Agency, Vienna, Austria; L. Roberts, U.S. Army Medical Research Unit, Nairobi, Kenya; W. Takken, UNDP/FAO, Maputo, Mozambique; and K. Bealby, Department of Veterinary and Tsetse Control Services, Mfuwe, Zambia. The financial support of the Overseas Development Administration, U.K. Government (Research Scheme R 3450), the Netherlands Foundation for the Advancement of Tropical Research (WOTRO), and U.S. Agency for International Development is gratefully acknowledged.

REFERENCES

ADE, E., HELMCHEN, G., and HEILIGENMANN, G. 1980. Syntheses of the stereoisomers of 17,21-dimethylheptatriacontane—sex recognition pheromone of the tsetse fly. *Tetrahedron Lett.* 21:1137–1140.

CARLSON, D.A., LANGLEY, P.A., and HUYTON, P. 1978. Sex pheromone of the tsetse fly: Isolation, identification, and synthesis of contact aphrodisiacs. *Science* 201:750–753.

CARLSON, D.A., LANGLEY, P.A., and COATES, T.W. 1981. Sex pheromones of *Glossina pallidipes*: Isolation, identification and synthesis. Proceedings of the 17th ISCTRC meeting, Arusha, Tanzania, Organization of African Unity, Nairobi, Kenya. In press.

COATES, T.W., and LANGLEY, P.A. 1982. The cause of mating abstention in male tsetse flies, *Glossina morsitans*. *Physiol. Entomol.* 7:235–242.

HELMCHEN, G., and LANGLEY, P.A. 1981. Bekämpfung der Tsetse-Fliege mit Pheromonen. *Nachr. Chem. Tech. Lab.* 29:294–298.

HUYTON, P.M., LANGLEY, P.A., CARLSON, D.A., and SCHWARTZ, M. 1980. Specificity of contact sex pheromones in *Glossina*. *Physiol. Entomol.* 5:253–264.

KOVATS, E. 1965. Gas chromatographic characterization of organic substances and the retention index system. *Adv. Chromatogr.* 1:229–247.

LANGLEY, P.A., PIMLEY, R.W., and CARLSON, D.A. 1975. Sex recognition pheromone in tsetse fly, *Glossina morsitans*. *Nature* 254:51–53.

LANGLEY, P.A., HUYTON, P.M., CARLSON, D.A., and SCHWARTZ, M. 1981. Effects of *Glossina morsitans morsitans* Westwood sex pheromone on behavior of males in field and laboratory. *Bull. Entomol. Res.* 21:57–63.

LANGLEY, P.A., COATES, T.W, and CARLSON, D.A. 1982a. A sex recognition pheromone in the tsetse fly *Glossina pallidipes*. *Experientia* 38:473–475.

LANGLEY, P.A., COATES, T.W., CARLSON, D.A., VALE, G.A., and MARSHALL, J. 1982b. Prospects for autosterilization of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), using sex pheromone and bisazir in the field. *Bull. Entomol. Res.* 72:319–327.

MCDOWELL, P.G., WHITEHEAD, D.L., and CHAUDHURY, M.F.B. 1981. The isolation and identification of the cuticular sex-stimulant pheromone of the tsetse *Glossina pallidipes* Austen (Diptera: Glossinidae) *Insect Sci. Appl.* 2:181–187.

MONDON, A. 1957. Der Aufbau von Olefinen aus 1,4-Dibromobutan nach der Methode von Wittig. *Annalen* 603:115–129.

NELSON, D.R. 1978. Long-chain methyl-branched hydrocarbons: Occurrence, biosynthesis, and function. *Adv. Insect Physiol.* 13:1–33.

NELSON, D.R., FATLAND, C.L., HOWARD, R.W., MCDANIEL, C.A., and BLOMQUIST, G.J. 1980. Re-analysis of the cuticular methylalkanes of *Solenopsis invicta* and *S. richteri*. *Insect Biochem.* 10:163–168.

NELSON, D.R., DILLWITH, J.W., and BLOMQUIST, G.J. 1981. Cuticular hydrocarbons of the house fly, *Musca domestica*. *Insect Biochem.* 11:187–197.

OFFOR, I.I., CARLSON, D.A., GADZAMA, N.M., and BOZIMO, H.T. 1981. Sex recognition pheromone in a west African tsetse fly, *Glossina palpalis palpalis*. *Insect Sci. Appl.* 1:417–420.

POMONIS, J.G., NELSON, D.R., and FATLAND, C. 1980. Insect hydrocarbons 2. Mass spectra of dimethylalkanes and the number of methylene units between methyl groups on fragmentation. *J. Chem. Ecol.* 6:965–972.

VALE, G.A. 1982. The improvement of traps for tsetse flies (Diptera: Glossinadae). *Bull. Entomol. Res.* 72:95–106.

VAN ETTEN, J. 1981. A comparison of the performance of laboratory colonies of *Glossina pallidipes* Austen from two allopatric populations in Kenya. *Insect Sci. Appl.* 1:177–183.